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Editorial

We are pleased to announce the second issue of Aurum Journal of Health Sciences (AJHS -A. J. Health Sci.) has been published. It consists of a case study from dentistry, a research paper to investigate antimicrobial effect of an extract, a research paper on essential oil composition of a plant, and a review article discussing the status of rare diseases and related orphan drugs in the world and Turkey. February 29 is celebrated as Rare Disease Day. The aim of this celebration is to raise awareness in society, and encourage further studies in the scientific field. It is known that although the name of the diseases is rare, their incidence is not rare. 6-7 million people only in Turkey are affected by these diseases. Therefore, supporting the researches in this field is also substantial in our country.

AJHS is published tri-annually as a peer-reviewed health sciences journal and aims to provide a platform for health science research with interdisciplinary discussions. Our vision is to contribute to publishing a journal taken part in national/international indexes. I received this mission from Assoc. Prof. Dr. Kaan Polatoğlu, who brought the journal to life with great effort, and I became the new chief editor. The way to be a significant journal in scientific fields begins with small steps. We can make this newborn journal grow into adulthood together. We are looking forward to hearing from our esteemed academicians to share their ideas, works and joint studies with us.

I would like to thank all of our editors who contributed to the issue of our journal, and to thank our referees and all those who contributed to the evaluation of articles. I wish a successful year for AJHS.

Gaye Hafez, PhD

Editor-in-Chief

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Case Study

Recurrence of Langerhans Cell Histiocytosis of Jaws: A Case Report with One Year Follow-up

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Abstract: Langerhans cell histiocytosis (LCH) is an unusual disorder characterized by high proliferation of Langerhans cells. A 27-year-old male patient was referred to the Oral Medicine Clinic, Faculty of Dentistry, Altınbaş University with a complaint of wound that does not heal for eight months. The clinical examination showed palatal mucosal ulceration extending to the bone at the upper right first molar region and ulceration at the lingual mucosa of the lower right first molar. The patient had been previously evaluated by a different dentist and was told that it was an aphthous lesion. A punch biopsy from the palatal mucosa and alveolar mucosa near the lesion revealed Langerhans cells and positive reaction to CD1 so the patient was diagnosed with LCH. The insignificant treatment trials may lead to diagnostic delay. Oral lesions may be the earliest symptom of LCH and in most cases; the oral cavity may be the only area involved. Awareness of lesions in the oral mucosa is important in achieving the accurate and early diagnosis and effective treatment plan.

Keywords: *Langerhans cell histiocytosis; histiocytosis X; jaw; recurrence*

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1. Introduction

Langerhans cell histiocytosis (LCH) is formerly called histiocytosis X, is characterized by abnormal proliferation of Langerhans cells (histiocytes), with varying numbers of lymphocytes, eosinophils, plasma cells, neutrophils, and multi-nucleated giant cells (Pacino et al., 1999). LCH was previously grouped into three clinical forms according to the patient age and the distribution of lesion including eosinophilic granuloma, Hand-Schüller–Christian disease, and Letterer-Siwe disease (Chu, 2001). The new classification

of LCH is made based on the dissemination of the disease and is grouped into two groups: nonmalignant diseases such as unifocal and multifocal eosinophilic granuloma and malignant diseases**,** including Letterer-Siwe disease and histiocytic lymphoma. LCH commonly occurs in young adults and children (White and Pharoah, 2014).

The oral symptoms may be the first sign of LCH and the oral cavity may be the only affected area in some cases (Shirley and Thomton, 2000). The gingiva and hard palate are the most commonly affected sites in maxillomandibular involvement. The oral symptoms of LCH include ulcerative and bleeding gingiva, mobile teeth, and pain (Altay et al., 2017). The ulceration is typically extended to the underlying bone in the oral mucosal involvement. LCH is known to look like different diseases and its diagnosis may be particularly difficult owing to its broad clinical spectrum, ranging from a single lesion to a multisystemic involvement. There are few reports about the misdiagnosis of this disorder (Chen and Peron, 2000; Kılıç et al., 2011; Zhang et al., 2006).

This case report defines a case of Langerhans cell histiocytosis with oral cavity involvement and presents the importance of the awareness of the oral manifestations of LCH.

2. Description of the Case

A 27-year-old male patient was referred to Altınbaş University, Dentistry Faculty, Oral Medicine Clinic with a complaint of palatal ulceration with eight months of duration. There was no pain or bleeding in the ulcerated areas. The patient had been previously evaluated by a different dentist and was told that it was an aphthous lesion. A mouthwash and wound gel was prescribed but the lesion did not regress. The patient had no significant systemic disease but he had a gonorrhea treatment a year ago. The right first molar tooth of the mandible was also extracted due to the periodontal abscess.

The clinical examination showed the necrotic area extending to the bone at the hard palate adjacent to the right first molar tooth (Figure 1a). The ulceration on the lingual mucosa and the necrotic area at the top of the alveolar crest was observed at the right first molar of the mandible (Figure 1b). Also the right and left submandibular lymphadenopathy were observed.

Radiologically, a periapical X-ray demonstrated vertical alveolar bone loss extending to the middle of mesial root of the second molar. The cone beam computed tomography (CBCT) was performed and it revealed the horizontal alveolar bone loss especially at the right premolar teeth region and the vertical alveolar bone loss at the mesial root of the right second molar tooth of the mandible. At the distobuccal root of the right first molar tooth of the maxilla, vertical alveolar bone destruction extending to the palatine bone was observed (Figure 2). Any other lesions in the remaining skull bones were not detected.

The punch biopsy from the palatal mucosa and alveolar mucosa was performed under local anesthesia and the specimen in 10% formal saline was sent for histopathological examination at the Department of Cytology and Tumor Pathology, Institute of Oncology, Faculty of Medicine, Istanbul University. This study was performed according to the guidelines of the Declaration of Helsinki concerning ethical

principles for medical research involving human subjects and written informed consent was obtained from the patient.

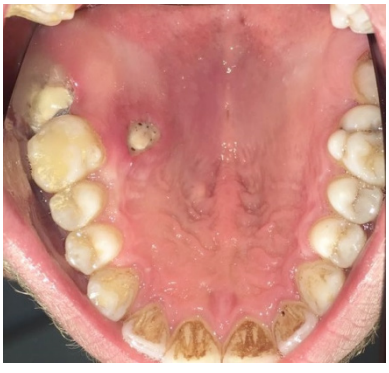
The histological and immunohistochemistry examination showed histiocytic cells, eosinophil polymorphs and positive reaction to CD1 (Figure 3a, 3b). According to these findings, the patient was diagnosed with LCH.

The patient was referred to the Department of Hematology, Faculty of Medicine, Istanbul University for further examination. The increased F-18 fluorodeoxyglucose (FDG) uptake was detected at the right first molar region both of maxilla and mandible and no other organ involvement was detected in the positron emission tomography (PET). The patient's clinical and radiologic findings were evaluated; it was decided to start chemotherapy treatment. The patient underwent chemotherapy for 6 sessions with Vincristine sulfate 10 mg but the treatment was not completed since the patient didn't show up for his sessions. The patient was admitted with a complaint of left femur pain after 6 months. The PET was performed and it revealed the hypermetabolic lytic lesion in the posterior ala of left iliac bone so radiotherapy was scheduled (Figure 4). The patient has applied 5 sessions of radiotherapy with the total radiation dose of 9 Gy iliac bone and 10 Gy tumor.

After the completion of radiotherapy treatment, in the follow up session in our department, the patient stated that the lesion in the mandible vanished after the first chemotherapy but the lesion at the palatal mucosa still not healed. It was seen as erythematous macular lesion with epithelized but incomplete keratinization and ill-defined margin (Figure 5). The patient is still under control 3-month intervals in department of hematology. Also he is asymptomatic for any other oral lessions with no further complaints of teeth mobility and pain.

Figure 1. a-b. The necrotic area extending to the bone on the right side of the hard palate (a). The ulceration on the lingual mucosa and the necrotic area at the top of the alveolar crest (b).

a)



b)

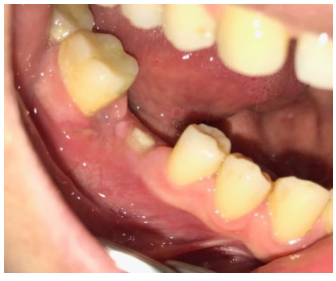


Figure 2. Axial view of CBCT, vertical bone destruction extending to the palatine bone at the distobuccal root of the right first molar tooth of the maxilla.

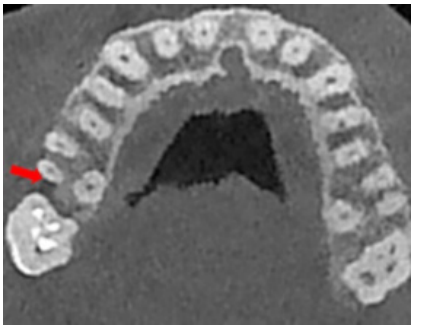
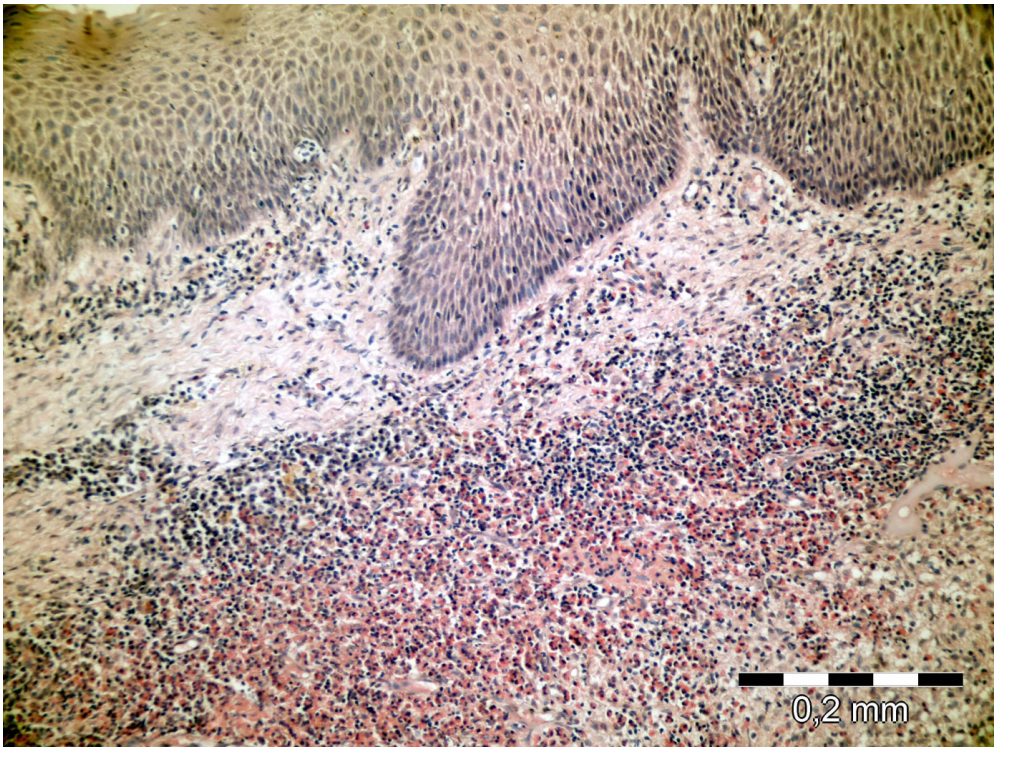


Figure 3. a-b. Langerhans cells and a large number of eosinophil polymorphs in the connective tissue (H&E X200) (a). Langerhans cells with strong positive staining by CD 1A primary antibody (CD1AX400) (b).

a)



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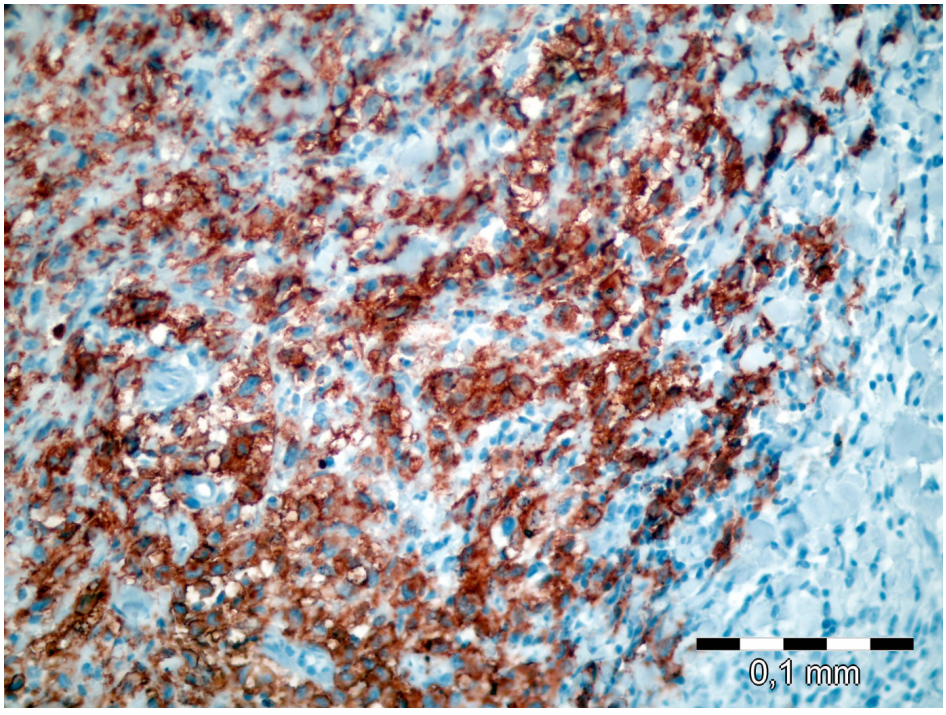


Figure 4. The increased FDG uptake at the posterior of left iliac bone

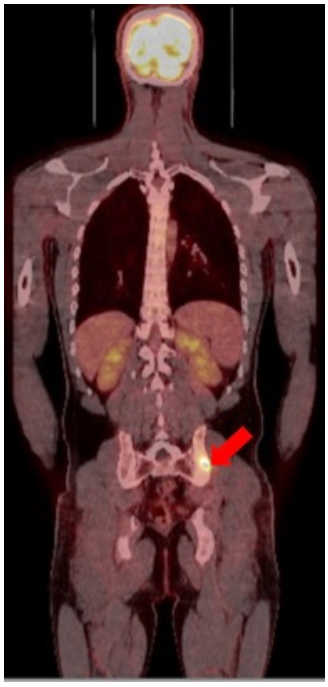
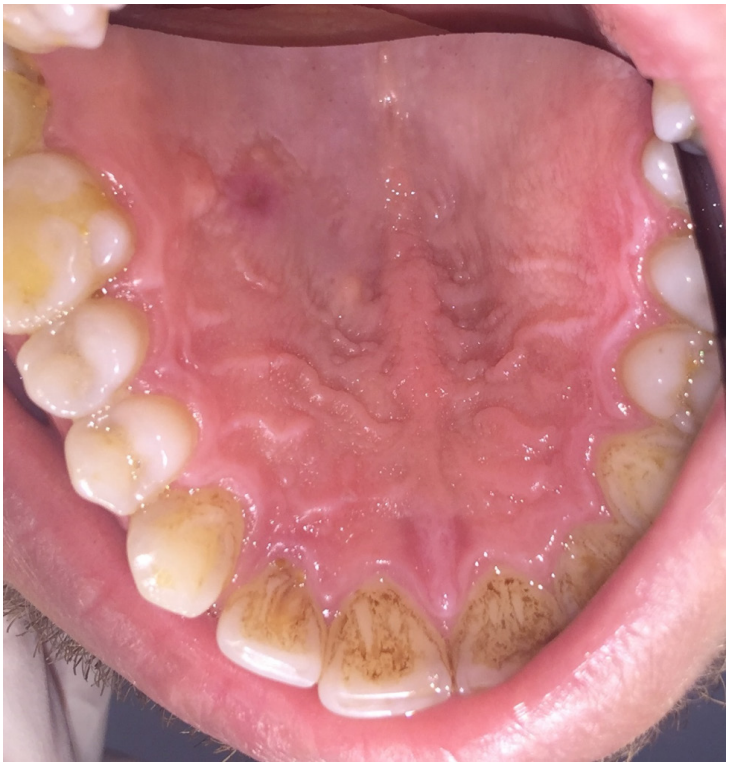


Figure 5. Erythematous macular lesion with epithelized but incomplete keratinization at palatinal mucosa.



3. Discussion

LCH is diagnosed by evaluating clinical and radiological findings and confirmed with the histopathological and immunohistochemical examination. Among the histopathological findings, the infiltration of Langerhans cells, eosinophilic granulocytes, lymphocytes and giant cells are considered to be characteristic of the disease. The characteristic immunophenotype of LCH includes positive reaction to CD1a, S100 protein and langerin (CD207) (Eckardt and Schultze, 2003; Merglova et al., 2014).

When encountered in the oral cavity, the differential diagnosis of LCH poses a major challenge to the dentist because many clinical features of the disease resemble the more common conditions, including periodontal disease, malignancies and granulomatous or ulcerative lesions (Chu, 2001). Chen and Peron (Chen and Peron, 2000) reported an eosinophilic granuloma case that was misdiagnosed as a radicular cyst in a 28-year-old patient. In our case the patient has evaluated misdiagnosis as an aphthous lesion clinically and insignificant treatment trials were performed. The pathologic examination of the present case was useful for early diagnosis, treatment, and prevention of serious complications. A correct evaluation of medical and dental anamnesis, clinical examinations, radiographic examinations, histological and immunohistochemical analysis essential to the achieve the accurate diagnosis.

In the prognosis, the early detection of LCH plays an important role which is closely related to the number of involved organs and the age of onset (Chu, 2001). This is especially important when the initial symptoms of LCH present in the oral cavity so it needs to be evaluated carefully. It is believed that the awareness of oral manifestations of LCH may help to clinicians greatly in reducing morbidity and mortality associated with this condition. Early diagnosis and effective treatment of LCH not only prevent the progression of the disease but also to prevent complications such as orthopedic impairment, skin scarring, hearing impairment, diabetes insipidus, and neuropsychological defects, liver cirrhosis, chronic pulmonary dysfunction, secondary malignancies (Bernstrand et al., 2007; Braier et al., 2002; Mittheisz et al., 2007; Nanduri et al., 2010).

The prognosis of LCH depends on the age at initial diagnosis as well as site and number of involved structures and organs. The treatment generally requires a multidisciplinary assessment. The rate of recurrence is 1,6-25 % (Koening, 2012). Abi-Akl et al. (Abi-Akl et al., 2017) reported a case which was previous history of LCH of the mandible, treated with radiotherapy and curettage and presented with recurrence in the temporal bone. In another case, Terada (Terada, 2013) reported a case which was previous history of LCH of the mandible, treated with surgical curettage and five years later presented with recurrence in the mandible and maxilla. In our case, the patient was admitted with a complaint of left femur pain after 6 months of chemotherapy and radiotherapy was applied. The patient is now asymptomatic and under control 3-month intervals in department of hematology.

The good integration of clinical, radiological examination, histological and immunohistochemical analysis may allow the clinician to reach the accurate diagnosis and form a multidisciplinary treatment plan. Oral lesions may be the earliest symptom of LCH and in most cases; the oral cavity may be the only area involved. Awareness of lesions in the oral mucosa is important in achieving the accurate and early diagnosis, effective treatment plan and prevention of complications.

Conflict of Interests

The authors declare that no conflict of interest

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Research Article

Determination of Antibacterial Effect of *Punica granatum* Shell Extract

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Abstract: *Punica granatum* (Pomegranate) is used as fresh fruit or processed products such as pomegranate juice, pomegranate sour and wine, jam. Although pomegranate is produced in our country, pomegranate shell is discarded without use. In recent studies; *P. granatum* containing rich phenolic compounds is known to have antibacterial, antifungal and antioxidant activity. The aim of this study was to investigate the antibacterial effect of *P. granatum* shell extracts prepared by ethanol, methanol and distilled water mixture on bacteria isolated from degraded cheese and salami samples by well diffusion method. In this study, identification of bacteria were performed by API biochemical identification test kits. API® 20 Strep for *Listeria monocytogenes*, API® 20E for *Salmonella typhimurium* and API® Staph test kits for *Staphylococcus aureus* were used. When the results of this study are evaluated, the zone diameters were measured as 18-24 mm and it was determinated that *P. granatum* shell extract has antibacterial effect against to isolated bacteria from salami and cheese samples.

Keywords: *Antibacterial activity; extract; inhibition zone; pomegranate; Punica granatum; well diffusion*

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1. Introduction

Today, it is known that food borne infections cause an increase in poisoning. On the other hand, it has been found that synthetic preservatives used in food production have a carcinogenic effect on human health. For this reason, producers have turned to the production of food preservatives which have antibacterial properties derived from natural products and which do not adversely affect human health (Al- Zoreky, 2009).

In recent years, the numbers of studies about identification of antioxidant and antimicrobial activities of natural compounds obtained from plants or fruits and their usage rate in the preservation of food products have been increased. Pomegranate (*Punica granatum*) shell is used as antimicrobial food

supplement or drug in many countries because of its high phenolic contents. It has been determined in some experiments that the phenolic compounds obtained from pomegranate shell have antibacterial, antifungal, antioxidant, antidiabetic and anticarcinogenic effects (Vuorela et al., 2005; Wang et al., 2010).

Studies of the effect of pomegranate on human health have shown that it strengthens the immune system, balances cholesterol and blood glucose value, protects against heart diseases and has anti-carcinogenic effect. In particular, it has been determined that phenolic compounds in *P. granatum* shell have antibacterial, antifungal, antiviral and anti-helminthic activity (Fischer et al., 2011; Gundogdu et al., 2011).

The aim of this study was to investigate the antibacterial effect of *P. granatum* shell extracts prepared by ethanol, methanol and distilled water mixture on bacteria isolated from degraded cheese and salami samples by well diffusion method.

2. Materials and Methods

2.1. *Preparation of Samples*

The bacteria were isolated that 25 g salami and cheese samples in expiration date past. The samples were homogenized in 225 mL buffered peptone water for pre-enrichment of bacteria. For the homogenization step, all samples were placed in a sterile polyethylene bag and it was shown in Figure 1. The samples were shaken for 2 minutes in Stomacher (Stomacher 400). $1.5x10^8$ CFU/mL (McFarland No: 0.5) bacterial suspensions were prepared in sterile distilled water and then a series of dilutions (10^8, 10^7, 10^6, 10^5 CFU/mL) were prepared to determine the number of bacteria. 100 µL of each dilution series were inoculated to Nutrient Agar (Oxoid). Each Petri dish was allowed to incubate at 37 °C. At the end of the period, bacterial colony counts were made.

Figure 1. Homogenization step of bacteria isolated from salami and cheese samples.

2.2. *Isolation of Salmonella typhimurium*

The homogenized samples in peptone water for the pre-enrichment stage were incubated at 37 °C for 24 hours. At the end of the time, 0.1 mL samples were inoculated into the tubes containing 10 mL of

Rappaport-Vassiliadis Broth (Merck) for selective enrichment and incubated at 42 °C for 24 hours. The samples were inoculated on to XLD (Xylose Lysine Deoxycholate) Agar medium (Merck) and incubated at 37 ° C for 24 hours. *S. typhimurium* suspected bacteria colonized on XLD Agar medium (red colonies with black centers) were evaluated according to Gram staining method and oxidase activity results. Gram and oxidase negative bacterial colonies were planted on Nutrient Agar (Oxoid) medium and API® 20E (Biomériux, France) test kit was used for the identification of suspected bacteria according to the manufacturer's instructions.

2.3. *Isolation of Staphylococcus aureus*

The samples homogenized in buffered peptone water for the pre-enrichment stage were incubated at 37 °C for 24 hours. At the end of the incubation period, 10^{-3} dilution series were prepared from the samples in Buffered Peptone Water for selective enrichment and 0.1 mL samples inoculated on to Baird Parker Agar (Oxoid) medium containing 5% Egg Yolk Tellurite. The samples incubated at 37 °C for 24 hours. At the end of the period, gray-black colored colonies were evaluated as suspicious for *S. aureus*. The samples were evaluated according to Gram staining, catalase, coagulase and oxidase activity. API® Staph (Biomériux, France) test kit was used for identification of *S. aureus* according to the manufacturer's instructions.

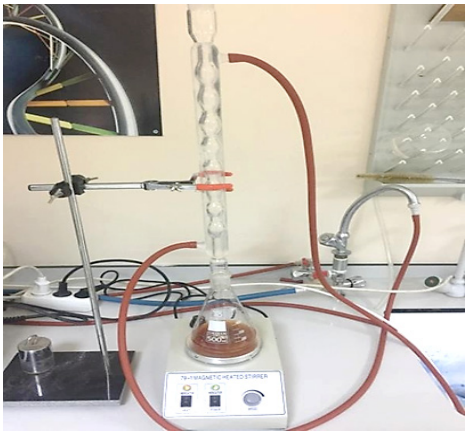
2.4. *Isolation of Listeria monocytogenes*

The samples homogenized in peptone water for the pre-enrichment stage were incubated at 37 ° C for 24 hours. At the end of the time, 0.1 mL of the samples in Buffered Peptone Water were inoculated into tubes containing 10 mL of Listeria Enrichment Broth (Merck) for selective enrichment and incubated at 37 ° C for 24 hours and then plated on Palcam Agar (Merck) medium. The samples were incubated at 37 °C for 24 hours. *Listeria monocytogenes* suspected bacteria colonized on Palcam Agar medium (gray-green, black zone, round colonies) were evaluated according to Gram staining method and oxidase activity results. Gram negative, oxidase negative bacterial colonies were plated on Nutrient Agar (Oxoid) medium and API® 20Strep (Biomériux, France) test kit was used for identification of suspected bacteria according to the manufacturer's instructions.

2.5. *Preparation of Punica granatum Shell Extract*

P. granatum shells brought to the laboratory were separated into small particles by using sterile lancet and the allowed to dry at room temperature. The dried specimens were pulverized by passing 3 times through a small-diameter sieve, and then 10 g of the sample was added to 100 mL of the mixture (70% ethanol, 70% methanol, distilled water). The mixture was placed in the evaporator and allowed to stand at 30 °C an hour at magnetic stirrer. It was shown in Figure 2. At the end of the incubation period, the homogenously mixed solution was passed through a 0.22 µm pore size injector filter (Millipore) to be sterile.

Figure 2. The evaporator used to obtain extract of *P. granatum* shell.



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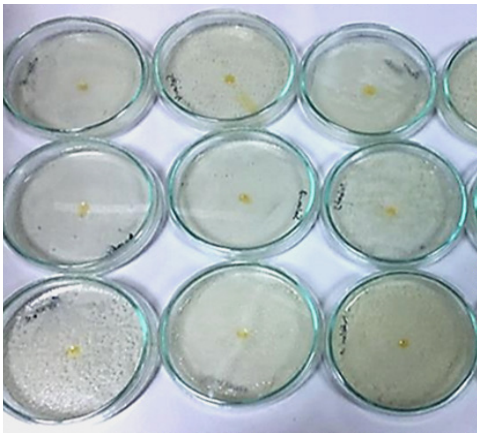
2.6. *Evaluation of Antibacterial Activity of Punica granatum Extract*

$1.5x10^8$ CFU/mL (McFarland No: 0,5) bacterial suspensions were prepared in sterile distilled water after identification of the bacteria isolated from salami and cheese samples by API biochemical test kit. 100 µL of the samples were inoculated on Mueller Hinton Agar (Merck) medium by spreading method. All of samples were incubated at 37 °C for an hour. A well diffusion method was used to evaluate the antibacterial effect of *P. granatum* shell extract (Al-Zoreky, 2009). For this purpose, the wells were opened into Mueller Hinton Agar (Merck) medium and 100 µL of *P. granatum* shell extract were inoculated to Mueller Hinton Agar medium. 100 µL of sterile PBS was used as a negative control. The samples were incubated at 37 °C for 24-48 hours. All of samples were run in 3 replicates. The inhibition zone diameters at the end of the time were measured in mm and the antibacterial activity of *P. granatum* shell extract was evaluated. It was shown in Figure 3.

Figure 3. The well diffusion method used to evaluation of antibacterial activity of *P. granatum* shell extract. A 1-3: *S. aureus;* B 1-3: *S. typhimurium*; C 1-3: *L. monocytogenes.*



The antibacterial of extract of *P. granatum* shell against bacteria isolated from salami and cheese samples will be measured inhibition zone diameters (mm). Then, the results were evaluated by comparing CLSI (Clinical & Laboratory Standards Institue) (2012).

3. Results

25 g of samples isolated from salami and cheese samples were transferred to Buffered peptone water for pre-enrichment stage. Then, suspected bacteria were spread on selective media and the appearance of the colony morphology on the media was observed and then the results of colony counts were shown in Table 1 and 2.

Table 1. The results of colony counts of bacteria isolated from salami samples.

		Serial dilutions (10^{-2}/10^{-5} CFU/mL)		
	10^{-2} CFU/mL	10^{-3} CFU/mL	10^{-4} CFU/mL	10^{-5} CFU/mL
Colony counts (CFU/mL)	1100	856	112	9

Table 2. The results of colony counts of bacteria isolated from cheese samples.

		Serial dilutions (10^{-2}/10^{-5} CFU/mL)		
	10^{-2} CFU/mL	10^{-3} CFU/mL	10^{-4} CFU/mL	10^{-5} CFU/mL
Colony counts (CFU/mL)	1310	744	98	14

The bacterial species identification of bacteria was performed by API biochemical identification test kits.

API® 20 Strep for *L. monocytogenes*, API® 20E for *S. typhimurium* and API® Staph test kits for *S. aureus* were used. Bacteria isolated from salami and cheese specimens were cultivated on Mueller Hinton Agar (Merck) media. After biochemical identification of bacterial specimens was done, well diffusion method was used to assess the antibacterial activity of *P. granatum* shell extract. The inhibition zone diameters (mm) were shown in Table 3.

When all of results obtained were evaluated, it was determined that the extract obtained from the *P. granatum* shell had antibacterial activity against bacteria isolated from salami and cheese samples. It was shown in Figure 4.

Figure 4. The antibacterial activity of extract of *P. granatum* shell.

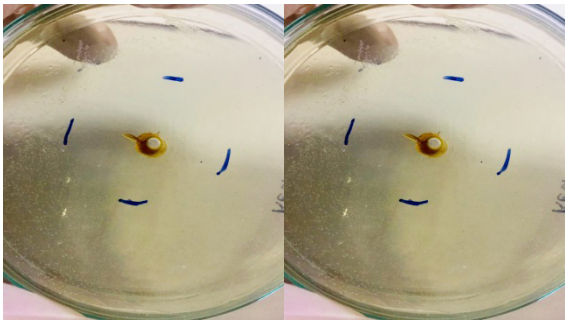


Table 3. The antibacterial effect of the extracts of *P. granatum* against bacteria isolated from salami and cheese samples.

Bacteria	Inhibition zone diameters (mm)	Average zone diameters (mm)
S. aureus	24 mm – 22 mm	23 mm
S. typhimurium	20 mm – 22 mm	21 mm
L. monocytogenes	18 mm – 18 mm	18 mm

4. Discussion

Because of the antimicrobial and antioxidant activity of the phenolic compounds in *Punica granatum*, the reconsidered to be effective in food preservation and as an alternative to synthetic food preservatives (Apaydin, 2008). It is thought that this product will extend the food preservation period and reduce the risk of food poisoning.

It has been determined that the extract obtained from the *P. granatum* shell (249.4 mg/L) contains much more phenolic material than the pulp extract (24.4 mg/L) (Tomas-Barberan and Espin, 2001). In the world and Turkey, pomegranate kernel sand shell is pressed in production of pomegranate juice. As a result of the pressing, there meaning portion is comprised approximately 73 percent of shell and 27 percent of kernel. The antioxidant and antimicrobial phenolic content of the shell part is higher than the other parts of the pomegranate juice; for this reason the work done in recent years has increased (Negi and Jayaprakasha, 2003; Li et al., 2006; Nuamsetti et al., 2012).

According to the studies done, the extracts obtained from pomegranate shell were detected to be effective in extending the shelf life of raw or cooked meat and poul try products (Naveena et al., 2008; Kanatt et al., 2010; Hayrapetyan et al., 2012). In our study, it was determined that the extract obtained from *P. granatum* shell was antibacterial effect against *Staphylococcus aureus*, *Salmonella typhimurium* and *Listeria monocytogenes*. According to this result, it is predicted that the shelf life of raw meat products will be extended.

In a study, when the antibacterial activity of *P. granatum* extractions grown in the Mediterranean Region was investigated, it was found to be effective against *Bacillus megaterium* DSM 32, *Pseudomonas aeruginosa* DSM 9027, *Staphylococcus aureus* Cowan 1, *Corynebacterium xerosis* UC 9165, *Escherichia coli* DM, *Enterococcus faecalis* A10 and *Micrococcus luteus* LA 2971 (Duman et al., 2009). In this study, the antibacterial activity of *P. granatum* shell extract was investigated after isolating bacteria (*S. aureus*, *S. typhimurium* and *L. monocytogenes*) from cheese and salami samples.

It was determined that *P. granatum* extract prepared with methanol had a strong antibacterial effect against *S. aureus* (32.3 mm) and *E. coli* (14.5 mm) (Shan et al., 2007). In other study, the antibacterial effect of extracts prepared from 46 medicinal plants were examined. It was determined that *P. granatum* had the highest total phenolic content compared to other plants and it was suggested that there was a direct correlation between antimicrobial activity and amount of phenolic material. In this study, it was determined that *P. granatum* extract had a strong antibacterial effect against *S. aureus* (23 mm), *S. typhimurium* (21 mm) and *L. monocytogenes* (18 mm) species isolated from cheese and salami samples.

In a study by Dahham et al. (2010), the inhibition zone diameter (25 mm) of *P. granatum* shell extract prepared with methanol against *S. aureus* and the result was found to be similar to the inhibition zone diameter (23 mm) against *S. aureus* in our study. In a study by Atya et al. (2018), the antimicrobial activity of extract of *P. granatum* at a concentration of 200 mg/ml was detected against *Pseudomonas aeruginosa* with a diameter of inhibition zone of 23.5 mm. Similarly, the diameter of inhibition zone was measured 22 mm against *S. aureus*, whereas 19.5 mm against *E.coli*, 17.5 mm against *Staphylococcus epidermidis* and 18 mm against *Staphylococcus saphrophyticus.* It was detected that the antimicrobial effect of *P. granatum* shell extract against *S. aureus* (Usman, et al. 2018). In this study, the diameter of inhibition zone was measured 13.67 ± 0.47 mm against *S. aureus*. In our study, the antibacterial activity of extract of *P. granatum* shell against *S. aureus* was observed to be 23 mm.

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Conflict of Interests

Authors declare no conflict of interests

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Research Article

Essential oil composition from aerial parts of *Scolymus hispanicus* L.

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Abstract: The volatile oil composition of *Scolymus hispanicus* L. was investigated. Essential oil of aerial parts were obtained through hydro-distillation and determined with GC-MS analyses. Fifteen compounds were determined in the oil (99.3%) of aerial part. The main compounds were heneicosane (19.4 %), hexahydrofarnesly acetone (17.0%) and phytol (17.0%). Saturated *n*-alkane derivatives (35.2%), oxygenated sesquiterpenes (25.6%) and diterpene (17.0%) were dominated in the oil. Also, the antibacterial activity of volatile oil was studied against *Escherichia coli* and *Staphylococcus aureus* bacteria. But the oil did not show activity against the tested microorganisms at 80-10 mg/mL concentrations. Here, it is reported for the first time on the volatile oil composition of *S. hispanicus*.

Keywords: *Scolymus hispanicus; volatile oils; heneicosan; hexahydrofarnesyl acetone; phytol*

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1. Introduction

Scolymus hispanicus L. (Golden thistle) is a member of Asteraceae family. *Scolymus hispanicus* is mainly found in Southern Europe and North Africa. There are three *Scolymus* L. species in Turkey (Davis, 1975). The plant has antisudorific and diuretic properties (Sari and Tutar, 2010). In Turkey, the root of the plant was used for kidney treatments between 1930-1990 years (Baser, 1993; Sari and Tutar, 2010; Sari et al., 2011). The plant also has been cultivated in Spain and Greece. There are a few research on the chemistry of *Scolymus hispanicus*. The methanol extract from aerial parts of *Scolymus hispanicus* was studied for the presence of phenolic compounds. The extract yielded one new flavonoid, six known flavonoids and four known phenolic acids (Sanz et al., 1993). The butanol extract from leaves of *Scolymus hispanicus* had two flavonol glycosides (Rubio et al., 1995). The flower extract of the plant included rosmarinic acid, orientin, quercetin 5-glucoside, and isorhamnetin 3-galactoside (Rubio et al., 1991). Also, nonacosane, α-amyrin, α-amyrin acetate, α-amyrin tetratriacontanoate, oleanolic acid, β-sitosterol, stigmasterol, fructose,

galactose, and mannitol were determined from the root bark of the plant (Erciyas and Baysal, 1989). The methanol extract of *Scolymus hispanicus* was investigated for antioxidant properties by different chemical assays. The extract showed strong antioxidant properties (Çetin, 2012). Taraxasteryl acetate was isolated from the ethanolic extract of the root bark of *Scolymus hispanicus*. The ethanolic extract and taraxasteryl acetate showed strong antispasmodic and spasmogenic activities (Kirimer et al., 1997). The aqueous-methanol extract of *Scolymus hispanicus* was studied on streptozotocin (STZ)-induced type 1 Diabetes Mellitus in rats as therapeutic potential. The extract remarkably improved fasting blood glucose level (Ozkol et al., 2013). The aerial part extracts (methanol and water) of *Scolymus hispanicus* were investigated for antiprotozoal and cytotoxic activities. Both extracts did not show any significant activity (Camacho et al., 2003). Fatty acid profiles of *Scolymus hispanicus* from Spain were studied. The main compounds of the plant were α-linolenic acid (30.55%), linoleic acid (26.44%) and palmitic acid (16.0%) (Morales et al., 2012). The total antioxidant capacity of 80% methanol extract of *Scolymus hispanicus* was studied by using CUPRAC, ABTS, FRAP and Folin assays. The extract showed a low total antioxidant capacity (Alpinar et al., 2009). Knowledge, use and ecology of *Scolymus hispanicus* from two localities in Central Spain were investigated. The result indicated that age and time living in the village showed differences in the knowledge and practice level (Polo et al., 2009).

There are no reports on the volatile oil composition of *S. hispanicus* in the literature. Here, it is reported for the first time on the volatile oil composition of *S. hispanicus*.

2. Materials and Methods

2.1. *Plant Materials*

Scolymus hispanicus was collected in İkitelli (Ziyagökalp)-Başakşehir, Istanbul, Turkey at 100 m altitudes on 27 June 2017 by Hüseyin Servi Ph.D. Identification of plant was done by Hüseyin Servi Ph.D. A herbarium specimen was kept in the Herbarium of Department of Pharmaceutical Botany, Faculty of Pharmacy, Marmara University (Herbarium no. MARE 18451).

2.2. *Isolation of the Volatile Oil*

The volatile oil of *Scolymus hispanicus* (400 g) was obtained by Clevenger apparatus (3 h) with hydrodistillation method. *S. hispanicus* aerial parts produced 0.04% (v/w) essential oil yields. The oil was kept with 1 mL *n*-hexane and hold in amber vials under -20°C till analyses day.

2.3. *Gas Chromatography-Mass Spectrometry Analysis*

The GC-MS analysis was employed with an Agilent 5975C Inert XL EI/CI MSD system in EI mode. Essential oil of aerial part was kept in *n*-hexane was injected (1 µL) in splitless mode. The temperatures of the injector and MS transfer line were adjusted at 250˚C. Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) and helium as carrier gas (1 mL/min) were utilized in GC/MS analyses. The temperature of oven was adjusted to 60˚C for 10 min. and increased to 220˚C at a rate of 4˚C/min. The temperature

kept stable at 220˚C for 10 min. and then increased to 240˚C at a rate of 1˚C/min. Mass spectra were saved at 70 eV with the mass range *m/z* 35 to 425. The relative percentage quantities of the separated compounds were calculated from integration of the peaks in MS chromatograms. The analysis was realized in triplicate.

2.4. *Identification of Essential Oil Components*

The determination of volatile oil compounds was realized by comparison with their relative retention indices got by a series of *n*-alkanes (C5 to C30) to the literature (Baser et al., 2000; Demirci et al., 2006; Demirci et al., 2013; Dregus and Engel, 2003; Kirimer et al., 2000; Kürkcüoglu et al., 2003) and with mass spectra comparison to the in-house libraries (Wiley W9N11, NIST11).

2.5. Antibacterial Activity

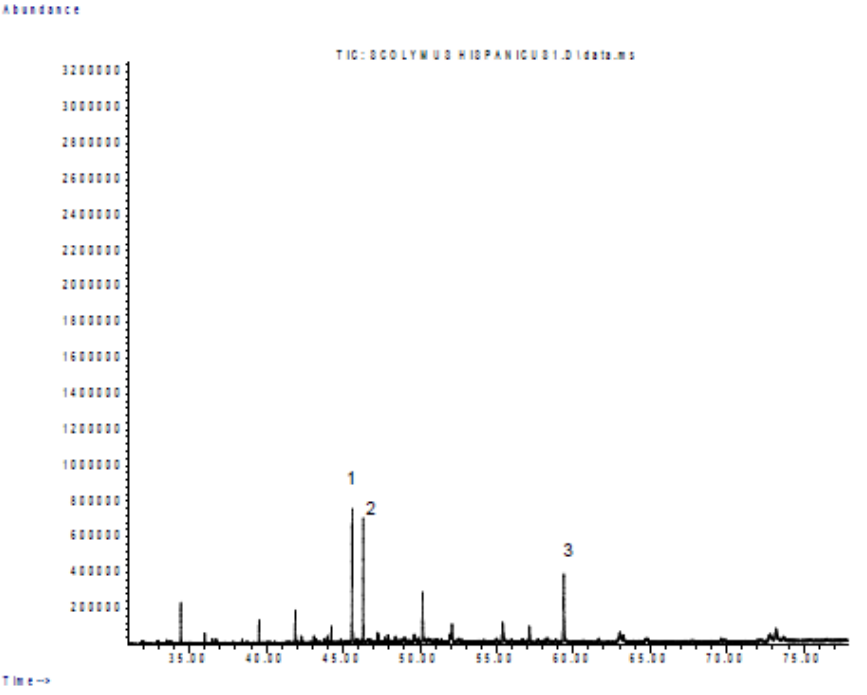
Antibacterial activity of the essential oil was studied against two strains; *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). Luria-Bertani broth was used as a growth medium for bacteria for the antibacterial tests.

In order to evaluate antibacterial activity, minimum inhibition concentration (MIC_{50}) values were detected by using the broth dilution method. Dimethylsulfoxide (DMSO) was used in the stock solution of volatile oil. The stock solutions were prepared on a 96 well plate as serial dilutions. After incubation at 37°C for 24 h, bacterial suspension concentrations were standardized to McFarland No:0.5. Volatile oil was mixed with bacterial cultures in the range of 1000-1,95 µg/mL as final concentration. It was paid attention to not exceed 1% final concentration for DMSO. After treatment, the bacteria were incubated at 37°C for 24 h. As a negative control, volatile oil-free solutions were utilized. Each test was repeated for three times. Growth analysis was done by using spectrophotometric measurements for MIC determination. Minimum inhibitory concentrations (MIC_{50}) were detected as the minimum concentration at which at least 50% of bacterial growth was missing.

3. Results and Discussion

Scolymus hispanicus aerial parts afforded 0.04% (v/w) amount of essential oils. Fifteen compounds were determined in the oil (99.3%) of aerial part. The main compounds were heneicosane (19.4 %), hexahydrofarnesly acetone (17.0%) and phytol (17.0%). Saturated *n*-alkane derivatives (35.2%), oxygenated sesquiterpenes (25.6%) and diterpene (17.0%) were dominated in the oil. Also, the antibacterial activity of volatile oil was studied against *Escherichia coli* and *Staphylococcus aureus* bacteria. The antibacterial activity of the oil evaluated with MIC values between 80-10 mg/mL. But the oil did not show activity against the tested microorganisms at 80-10 mg/mL concentrations.

Figure 1. GC-MS Chromatogram of *Scolymus hispanicus* essential oil.



1: Heneicosane; **2:** Hexahydro farnesyl acetone; **3:** Phytol.

Table 1. The volatile oil composition of *Scolymus hispanicus*

RRI[1]	RRI Lit.[2]	Compound	I[3] (%)	II (%)	III (%)	Average[4] (%)	SD[5]	Identification method[6]
1680	1687	Estragole	5.6	5.7	5.3	5.5	0.2	RI, MS
1733	1737	β-Bisabolene	1.4	1.4	1.3	1.4	0.1	RI, MS
1861	1864	*Trans*-geranyl acetone	3.6	3.5	3.3	3.5	0.2	RI, MS
1951	1958	*Trans*-β-ionone	4.7	4.6	4.4	4.6	0.2	RI, MS
2044		*Cis*-davanone	2.5	2.3	2.4	2.4	0.1	MS
2100	**2100**	**Heneicosane**	**20.0**	**19.5**	**18.6**	**19.4**	**0.7**	RI, MS, Ac
2131	**2131**	**Hexahydro farnesyl acetone**	**16.4**	**16.6**	**18.1**	**17.0**	**0.9**	RI, MS
2300	2300	Tricosane	7.7	7.6	7.4	7.6	0.2	RI, MS, Ac
2374	2380	α-Hexyl cinnamaldhyde	1.7	1.6	1.5	1.6	0.1	RI, MS
2380	2384	Farnesyl acetone C	3.3	3.3	1.2	2.6	1.2	RI, MS
2501	2500	Pentacosane	5.2	5.2	5.1	5.2	0.1	RI, MS, Ac
2551	2592	Diisobutyl pthalate	3.7	3.6	3.6	3.6	0.1	RI, MS
2614	**2622**	**Phytol**	**17.3**	**17.1**	**16.5**	**17.0**	**0.4**	RI, MS
2701	2700	Heptacosane	2.9	3.1	3.1	3.0	0.1	RI, MS, Ac
2909	2931	Hexadecanoic acid	4.0	5.0	6.2	5.1	1.1	RI, MS
		Oxygenated sesquiterpene	25.8	25.9	25.0	25.6		
		n-alkane derivatives	35.8	35.4	34.2	35.2		
		Diterpene	17.3	17.1	16.5	17.0		
		Fatty acid and esters	4.0	5.0	6.2	5.1		
		Monoterpene	3.6	3.5	3.3	3.5		
		Others	13.5	13.2	12.8	13.2		
		Total	**100.0**	**100.0**	**98.0**	**99.3**	**1.2**	

[1]RRI: Relative retention time; [2]RRI Lit.: Relative retention time in the literature; [3]The analysis results; [4,5]The average % area of analysis with ± standard deviation (SD); [6]Identification method.

According to recent genetic analyses, the genus *Scolymus* is related to with some genus such as *Gundelia*, *Hymenonema*, and *Catananche* (Liveri et al., 2016). Previously, essential oils with high content of thymol (11.2%), γ-terpinene (9.8%), germacrene D (6.6%) and *p*-cymene were reported for *Gundelia tournefortii* from Iran (Dastan and Yousefzadi, 2016). And an another study from Iran, palmitic acid (12.48%), lauric acid (10.59%), α-ionene (6.68%), myristic acid (4.45%), 1-hexadecanol,2-methyl (3.61%), phytol (3.6%), and

β-turmerone (3.4%) were major components of volatile oil of *Gundelia tournefortii* (Farhang et al., 2016). Additionally, essential oils of two varieties of *Gundelia tournefortii* from Turkey were studied. The main compounds were determined thymol (24.5%) in *G. tournefortii* var. *tournefortii* oil, germacrene D (21.6%) in *G. tournefortii* var. *armata* oil (Bağcı et al., 2010). The main compounds of *Scolymus hispanicus* were not detected in the oil of *Gundelia tournefortii* from Iran and Turkey (Dastan and Yousefzadi, 2016; Bağcı at al., 2010). But these main compounds were contained low amounts in the oil of *Gundelia tournefortii* from Iran and the results of the essential oil analysis of two species, some similarities observed in their compositions (Farhang et al., 2016).

Conclusion

The volatile oil composition of *S. hispanicus* from Turkey was studied for the first time. There is no research on the volatile oil of *Scolymus* genus, that's why it is hard to give a comment on the chemo-systematic situation of *Scolymus hispanicus* depending on the present study. The results will help further research on the chemistry of *Scolymus* genus.

Conflict of Interests

Author declares no conflict of interests.

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Review

Rare Disease and Orphan Drug Situations in Turkey and around the World

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Abstract: Diseases with a prevalence less than 1/2000 are defined as "Rare Diseases". This group of diseases is a highly heterogeneous group that affects multiple systems. Approximately 80% of these are caused by genetic reasons, and the remaining 20% are caused by environmental factors or are idiopathic. Orphan drug is defined as a medicinal product designed for a life-threatening or chronically impairing rate disease but with a low possibility of obtaining a return on investment due to insufficient sales. In Turkey, where consanguineous marriage frequency is 25% on average, the probability of rare diseases is quite high. Lack of knowledge and specialist physicians, very expensive treatments, and lack of a designated regulation for rare diseases and orphan drugs are the main problems in our country. The research revealed that orphan drugs were imported to Turkey through the Turkish Pharmacists' Association (TEB) and not covered by Social Security, therefore making it hard for the patients with low income to reach these drugs.

Keywords: *Rare; disease; orphan; drug*

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1. Introduction

"Rare diseases" are occurred in a small number of people in comparison with the general population and basically the biggest problem of these diseases is they are thin on the ground. In Europe, diseases encountered in 1 person in 2000 are considered rare. A disease may be rare in an area while it may be frequent in another (http://www.orpha.net accessed 2016).

Rare diseases are highly heterogeneous group that affects any or multiple systems. Approximately 80% of these are caused by genetic reasons, and the remaining 20% are caused by environmental factors or are idiopathic. They progress with severe physical-mental impairments. These impairments negatively affect the quality of life and the life expectancy of the patients is quite low. Although rare diseases show

different epidemiological characteristics from country to country, they constitute an important public health problem for each country and lead to special diagnostic, treatment and follow-up difficulties. Therefore, these diseases require special approaches and applications, and they should be handled separately from common diseases (Dündar and Karabulut, 2010).

Rare diseases are medically important public health problems. These diseases can be described as "health orphans", in other words, "Orphan Diseases". "Orphan" is a Greek word. Calling these diseases which have been neglected for many years, have been hard and costly to research, diagnose, and treat "Orphan Diseases" is not a wrong analogy (Dündar and Karabulut, 2010).

The number of rare diseases also depends of the accuracy of the designation of the factors constituting the disease. Medical field designated "disease" as a change that occurs as a unique symptom pattern in the health status of an individual and that has a single treatment. Whether the pattern is unique or not is completely based on the accuracy of our analysis. This confusion is reflected in certain classifications of Orphanet (http://www.orpha.net accessed 2016).

2. Rare Diseases

Almost all genetic diseases are rare diseases; but not all rare diseases are genetic. For example, there are very rare infectious diseases, as well as autoimmune diseases and rare cancers. Today, the cause of many rare diseases is unknown. Rare diseases are severe, mostly chronic and progressive diseases. In many rare diseases, as in proximal spinal muscular atrophy, neurofibromatosis, brittle bone disease, chondrodysplasia or Rett syndrome, the symptoms may be observed at birth or in childhood. However, more than 50% of rare diseases (such as Huntington's disease, Crohn's disease, Charcot-Marie-Tooth disease, amyotrophic lateral sclerosis, Kaposi's sarcoma, or thyroid cancer) manifest during adulthood (http://www.orpha.net accessed 2016).

In our country, where consanguineous marriage is common, the high number of rare diseases has grabbed the attention of genetic researchers. According to WHO data, 1:1000 people in every 100.000 people in European countries suffer from rare diseases. The prevalence of rare diseases is higher in Turkey due to consanguineous marriages. While the ratio of consanguineous marriages in the EU is 3-10 in a thousand, the ratio of consanguineous marriages in Turkey is 12-17 in a hundred. Therefore, it is expected that approximately 5-7 million people are affected by rare diseases in Turkey. Besides, our country also experiences the problems encountered throughout the world such as lack of knowledge about Rare Diseases and lack of specialist physicians in these areas, the high costs of the treatment of these diseases and the lack of a regulation that designates Rare Diseases and Orphan Drugs (Özbek U, 2014).

2.1. *The Prevalence of Rare Diseases*

In 2001, it was determined that the number of rare diseases was around 5000 and it constituted 10% of all human diseases. Today, this number is increasing day by day, and about 4-5 new diseases are

designated every month (Campos-Castell, 2001). There are between 5000 and 8000 rare diseases; most of them are of genetic origin and are noticed in childhood. They have high mortality and morbidity, and cause chronic debilitation in patients. It is thought that 6,5/10.000 people suffer from rare diseases in the Unites States (USA). Additionally, the Community Action Program between 1999 and 2003 showed that the prevalence was 5/10,000 in the European Union countries (Dear et al., 2006; Stolk et al., 2006; Taruscio and Cerbo, 1999).

Although this may seem like a low number when considered individually, considering the high number of these diseases, the fact that it is a major public health problem with the sheer number of people affected in the European Countries such as Turkey and Italy, and the United States should not be forgotten. Therefore, it will be obvious that the number of the affected individuals is remarkably high. Different sources report that the number of people affected by rare diseases is 30 million in Europe and 25 million in North America (Wastfelt et al., 2006).

80% of the rare diseases are neuropathic diseases and the majority of these are diagnosed in childhood (Campos-Castell, 2001). There are neurological disorders in more than half of these, but there is no preventive or therapeutic approach. The prevalence evaluation of rare diseases is carried out by European Organization for Rare Diseases (Eurordis) and Orphanet with the support of the European Commission. The examples of these diseases include pulmonary arterial hypertension, Fabry disease, hereditary angioedema, chronic myeloid leukemia, idiopathic pulmonary fibrosis, cryopyrin-associated periodic syndrome (CAPS), gout, Familial Mediterranean Fever (FMF) (http://www.orpha.net accessed 2016).

2.2. *What are the medical and social consequences of the rarity of these diseases?*

There is a lack of medical and scientific knowledge in the rare diseases field. Physicians, researchers and political authorities have long been unaware of rare diseases and until recently. For most rare diseases, there is no total cure for the disease; however, appropriate treatment and medical care may improve the quality of life of patients and prolong their life expectancy. Striking advances in certain diseases indicate that we should continue to fight in the research and social solidarity areas (http://www.orpha.net accessed 2016).

All of those affected by such diseases face similar difficulties in accessing relevant information and qualified specialists in addition to the diagnostic process. Access to quality health care, general social and medical support, effective communication between hospitals and general practices, as well as professional and social integration and independence have emerged as equally specific problems (http://www.orpha.net accessed 2016).

Patients affected by rare diseases are also weaker in psychological, social, economic and cultural terms. These challenges can be overcome with appropriate policies. Most patients cannot be diagnosed due to the lack of adequate scientific and medical knowledge. The diseases of these patients are not designated. These are the people who have the greatest difficulty in getting the appropriate support (http://www.orpha.net accessed 2016).

2.3. *Rare Diseases Designation Problem*

Rare diseases are often designated late. This leads to significant problems in patients in terms of survival. For example, a questionnaire sent to 18,000 people composed of organizations and patients in 17 European countries regarding rare diseases including Crohn's Disease, Cystic Fibrosis, Duchenne Muscular Dystrophy, Ehlers-Danlos Syndrome, Marfan syndrome, Prader-Willi Syndrome, Tuberous Sclerosis, and Fragile X Syndrome revealed that 25% of the patients were diagnosed with correct diagnosis 5-30 years after the first symptom. Before definitive diagnosis, 40% of the patients were followed up with a misdiagnosis and 60% were not even diagnosed. Most of the misdiagnosed patients have been subjected to unnecessary medical interventions. 16% were operated unnecessarily, 33% received inappropriate medical treatment and 10% received psychological treatment with the assumption that the disease was psychosomatic. 25% of the patients went to another center to confirm the treatment and 2% applied to other centers abroad. One third of the patients did not find the communication methods established to make a diagnosis satisfactory. Therefore, this multi-centric and large scaled study showed that the knowledge and medical expertise required to diagnose rare diseases is insufficient. Therefore, the possibility of complications and late sequel is very high in these patients who have already been delayed more than enough. A prospective study conducted in Italy (between 1985 and 1997) shows that only 19,5% of 1935 infants born with metabolic disease can reach adult age (Dionisi-Vici C et al., 2002).

In order for the patients to access specialist care centers, the need to strengthen the links between reference centers should be a priority for many countries. The European Commission experts have proposed to improve the diagnostic tests of rare diseases and to reduce and prevent rare genetic diseases with early diagnosis with the universal neonates screening in European Union member countries. There are reference centers specialized in rare diseases, but most patients are not even aware of the existence of these centers (Dündar and Karabulut, 2010).

2.4. *International Organizations*

Rare Diseases cover a highly heterogeneous group, both prevalently and medically. Knowledge about these diseases, diagnostic methods and treatment options are also heterogeneous. Low prevalence, geographic distribution of patients and researchers cause a lack of infrastructure related to these diseases. This has revealed the need for the establishment of various funds. France, Germany, Italy and Spain are conducting a research program that supports the interdisciplinary in which the resources related to rare diseases are insufficient and international information network (Wetterauer and Schuster, 2008).

For the last 25 years, various authorities have noticed that the medical processes, which are diagnosis, prevention and treatment alternatives, are quite backward compared to normal diseases. Additionally, the pharmaceutical industry has not been willing to support drug development projects related to these diseases. With the increase in national awareness, support organizations related to this situation were also established abroad. "The National Organization of Rare Disorders (NORD)" founded in 1983 is an example from the United States (Dündar and Karabulut, 2010).

"Eurodis" is an organization established in Europe in 1997 taking NORD as a model to improve the quality of life of people affected by rare diseases. It has also been a driving force for the adoption of the European Regulation on Orphan Drugs. Therefore, the organizations supporting these diseases, in particular NORD, pressured the US authorities in 1983 and the Orphan Drug Act, which was later adopted by other countries, was enacted. Thus, in 1983, the US-The Orphan Drug Act, in 1993 Japan, and in 2006, the European Union enacted various laws related to the treatment and prevention of these diseases (Haffner, 2008).

The International Classification of Diseases (ICD) coding system used by many countries is not suitable for rare diseases. Lack of an international coding system for these diseases leads to the lack of a common knowledge bank, and a language for the prevention of these diseases, protection methods and treatments for both patients and researchers of these diseases. This is an extra problem for these diseases, which are already in the second place because of the small number of them and because their treatment significantly affects the country's economy. To this end, national and international organizations have been established for some rare diseases. Although these organizations vary from country to country, they are usually established under the leadership of researchers, patients, public institutions and pharmaceutical companies (Dündar and Karabulut, 2010).

3. Orphan Drug and Related Designations

According to the WHO definition, "Drug is the substance or product that is used/intended to be used to change the physiological systems in favor of the pathological conditions field". Before a drug is introduced, it is subjected to extensive research and strict controls, and it is made available only after it is approved by the authorities and licensed (Akıcı and Ulupınar, 2013).

There are other features that distinguish a drug from other products. The facts that drugs are included into life with a very dynamic and detailed knowledge and they are produced and consumed, they are subject to comprehensive legislation, they have rigorous pre-clinical and clinical research process before licensing, licensing process, after-license research, review and surveillance processes, pricing, reimbursement processes, and equivalents, the patent period, the struggles to stay in the market among competitors, and management of crisis in health and non-health related issues are examples of this privileged position of drugs (Republic of Turkey Social Security Institution Book, 2013).

While new drugs based on a patented molecule and does not have any similar are considered as "original drugs", and the products that are proven by the scientific studies that they have the same properties with the original drugs and provide the same treatment, but that can only be launched after the expiration of the patent periods of the original drugs are called "generic (equivalent) drugs" (Çalışkan, 2008).

"Orphan" drugs are drugs that the sponsors are reluctant to develop under normal marketing conditions since the small market-size of the drugs that are meant to treat but address to really rare diseases will not allow the sponsors to pay off the capital they invest in the research and development of the product (http://www.orpha.net accessed 2016).

Patients affected by rare diseases cannot be excluded from treatment options and developments in science; they have equal rights with other patients in terms of treatment. Public authorities have introduced incentive measures for the health and biotechnology industry to trigger research and development activities in the orphan drug sector (http://www.orpha.net accessed 2016).

These practices started with the enactment of the Orphan Drug Act in the United States in 1983, followed by the practices in Japan and Australia in 1993 and 1997; and the practices started in Europe in 1999 when Member States adopted the orphan medicine policy (http://www.orpha.net accessed 2016).

The special care and attention required for these drugs which the pharmaceutical industry is reluctant to develop introduced the "orphan drug" concept. A drug is called an orphan drug only when there is a scientific justification suitable for its use during any stage of the use of this drug (Blankart et al., 2011).

Patients affected by rare diseases cannot be excluded from treatment options and developments in science; they have equal rights with other patients in terms of treatment. Public authorities have introduced incentive measures for the health and biotechnology industry to trigger research and development activities in the orphan drug sector (Blankart et al., 2011).

Access to orphan drugs is important to reduce the morbidity and mortality of rare diseases. For example, until pirfenidone was introduced, lung transplantation was the only treatment option for patients with idiopathic pulmonary fibrosis and survival was 3 years with 50% chance (Aagaard and Kristensen, 2014). Although the suitability and availability of orphan drugs are important and necessary, there are challenges in the treatment of these diseases. Such that only one out of 10 patients with rare disease receives special treatment. The development of orphan drugs requires high costs and original investments, given the small patient population per rare disease (Blankart et al., 2011).

The development of orphan drugs often follows the same legislative pathways as other drugs because of the pharmacokinetic, pharmacodynamic, dosing, stability, safety and efficacy tests required to be performed. However, certain statistical responsibilities have been reduced in their development. For example, orphan drug regulations agree that phase III clinical trials cannot be performed on 1000 patients in the development of drugs. The market area of the drugs with limited application can be quite small, so it is not considered profitable for companies. Health Authorities carry out motivating activities in this sense (Hadjivasiliou, 2014). Governments may have interventions in various areas of drug development:

- Tax deduction
- Improved patent protection and marketing rights
- Providing financial assistance for clinical trials
- Providing a government-sponsored initiative for research and development

Orphan drug manufacturers also benefit from a small customer base to reduce costs on the clinical side, as they have smaller trials. According to the FDA, the approval period for orphan drugs for clinical trials is 10 months while this period is 13 months for normal drugs. This allows the orphan drugs to be introduced to the market as soon as possible. A 2011 study revealed that orphan drug trials between 2004 and 2010

were smaller and with higher probability for less random selection compared to non-orphan drugs, but still had a higher FDA approval rate, and 15 orphan cancer drug was approved while this number was 12 for non-orphan cancer drugs (Kesselheim et al., 2011).

The 2014 orphan drug report shows that the orphan drug designations continue to increase rapidly. It is expected that the prescribed orphan drugs sold until 2020 will be $176 billion. Although the orphan populations are small, the cost of spending per patient is quite high. The same report emphasized that orphan drug economy mirrored the entire pharmaceutical market but there were some big differences (Hadjivasiliou, 2014).

Although the European Medicines Agency (EMA) allows access to all member states, in practice, each Member State enters into the market with the drugs which will be paid by its own national health system. For example, 35 orphan drugs were introduced to market in Belgium, 44 drugs in the Netherlands, 28 drugs in Sweden in 2008, 35 drugs in France and 23 drugs in Italy in 2007 (Denis et al, 2010).

The fact that a drug has been authorized for marketing in Europe or America does not mean that this drug is available in all countries. The marketing authority holder must decide in advance on the commercialization in each country; the drug then undergoes the necessary procedures in each country to determine the conditions of reimbursement and the price. The approach differences between the countries in spite of the joint efforts further complicate patients' access to orphan drugs (European Medicines Agency, https://www.ema.europa.eu/en/human-regulatory/overview/orphan-designation-overview (accessed 15 October 2016).

3.1. *Orphan Drug Development in the USA*

In the United States of America, pressure and lobbying activities were carried out by the National Rare Disorders Organization to have a specific law that would encourage pharmaceutical companies to develop orphan drugs. Within this context, in 1979, the FDA established a Task Force on orphan diseases: "When a drug is designated as a potentially life-saver or otherwise providing unique great benefit to a patient, the search and the introduction of this drug by the government is the obligation of the community represented." Thus, in January 1983, the Orphan Drug Act was adopted in the USA. The purpose of the FDA Office of Orphan Products Development is to evaluate and develop the products (drugs, biological products, devices or medical foods) intended for the diagnosis and treatment of rare diseases or conditions. In carrying out this task, the FDA evaluates all the scientific and clinical data obtained from companies in order to design and designate the promised products for rare diseases and to further the scientific development of promising medicinal products (Pedro, 2013).

The Office of Orphan Products Development provides incentives for companies to develop orphan drugs. Since 1983, this program has successfully enabled the development and introduction of more than 575 biological products and drugs for rare diseases. However, less than 10 products supported by the industry were introduced to the market between 1973-1983. There are also other programs that deal with orphan medicines in the USA. The Orphan Drug Designation program provides orphan status to drugs and biological origin designed for safe and effective treatment, diagnosis or prevention of rare

diseases/disorders affecting more than 200,000 people in the USA. However, it is not expected to provide for the development and marketing costs of the drugs that can provide treatment. The Rare Pediatric Disease Priority Review Voucher Program states that a sponsor that was granted approval for a drug or biological product for a "rare pediatric disease" may be eligible to receive a certificate that can be used to have a future marketing application examined with priority. Humanitarian Device Exemption (HDE) Program states a device that intends to benefit patients by treating or diagnosing a disease or condition that affects fewer than 4,000 new people in the United States each year. With the increase in national awareness about rare diseases and orphan drugs in the USA, support organizations have started to be established. For example, there are international organizations in the USA, such as the National Organization for Rare Disorders (NORD) founded in 1983. These groups are focused on improving the treatment of rare diseases. Patients often participate in these organizations to trigger and create a pressure on prescribers, regulatory agencies and political bodies in relation to the availability of orphan drugs. There are patient advocacy groups for easier access to orphan drugs and their treatment, and these groups lobby for third party taxpayers or governments that finance health care, to ensure full reimbursement of orphan drugs, regardless of their higher prices. In addition to the authorities, some universities leap forward on the subject. For example; the Orphan Drug Research Center at the Pharmaceutical University of Minnesota helps small-scaled companies with insufficient expertise and resources in drug synthesis, formulation, pharmacometry and bio-analysis. The Center for Rare Disease Therapies at the Keck Institute in Claremont, California, supports existing projects to stimulate the inactive potential orphan drugs by identifying barriers to commercialization, such as formulation and problems with biological processing (Developing Products for Rare Diseases & Conditions. U.S. Department of Health and Human Service http://www.fda.gov/orphan/oda.htm; accessed 7 February 2010).

In 1989, the Good Therapeutic Act was amended to include certain articles that would encourage Australian pharmaceutical companies to develop orphan drugs. However, the full orphan drug policy was established in 1997 (Gammie et al, 2015).

3.2. *Orphan Drug Development in the European Union*

The European Union defines a rare disease as a condition that threatens life or chronically weakens maximum 5 people in 10,000 people (Pedro, 2013).

A decision adopted by the EU Council of Ministers on November 30, 1995 requested the European Commission to investigate rare diseases and consider certain legislation on orphan drugs. The Orphan Medical Products Regulation (EC) N° 141/2000 was adopted by the European Parliament and the European Council on December 16, 1999. Additionally, the European Commission defined "similar medicinal products" and "clinical superiority" terms and introduced application provisions on orphan drugs and adopted the Regulation (EC) N° 847/2000 on April 27, 2000. Pursuant to the European regulation n ° 141/2000, only human drugs can be called "orphan drugs". Therefore, this definition does not cover veterinary medicines, medical devices, nutritional supplements and dietary products (http://www.orpha.net accessed 2016).

Additionally, with this Regulation, the European Commission adopted a new Regulation (847/2000) stipulating the provisions for the application of the criteria which are important in the designation of orphan drugs, the definition of similar medicinal products and the clinical superiority. The aforementioned regulations are the regulatory key framework for orphan drug provisions in the EU (Pedro, 2013).

The European Union has a central procedure for orphan drug designation and approval of marketing among its member countries. Cross-border regulations are of paramount importance since patients cannot be treated due to inadequate access to orphan drugs and the lack of specialized investigators and facilities. The 2011/24/EC Directive clarifies patient rights in cross-border health care. This directive enables the patients with rare diseases in the Member States to get health care services throughout the European Union in case the national health system cannot provide the necessary treatment within a reasonable period of time. However, due to differences in national pricing and reimbursement policies among Member States of the European Union, the patients still suffer from different access to orphan drugs (Aagaard and Kristensen, 2014).

Patients often participate in certain organizations to trigger and create a pressure on prescribers, regulatory agencies and political bodies in relation to the availability of orphan drugs. In Europe, for example, the Rare Diseases Europe (EURORDIS) was established focused on improving the treatment of rare diseases. Patient advocacy groups can establish partnerships with regulatory bodies such as EMA and EURODIS (Gammie et al, 2015).

4. Orphan Drug Status in Turkey

In our country, where consanguineous marriage is common, the high number of rare diseases has grabbed the attention of genetic researchers. According to the World Health Organization data, 8,000 people in every 100,000 people in European countries suffer from rare diseases. The prevalence of rare diseases is higher in Turkey due to consanguineous marriages (Dündar and Karabulut, 2010).

A study carried out on 55,175 marriages between the years 1970 to 1987 revealed that consanguineous marriage rate in Turkey was 21.21%, and that most of them was the first-degree cousin marriages. While consanguineous marriage rate is 3-10 per thousand in European Union countries, this rate is higher in Turkey. The rate of consanguineous marriages in different regions varies between 20-25%. The incidence of autosomal recessive diseases in the children born to these marriages is found to be above the average of Turkey. Therefore, it is expected that approximately 5.7 million people are affected by rare diseases in Turkey. The rate of consanguineous marriages differs according to race, social characteristics, religion and moral rules. A study investigated the effects of consanguineous marriages on miscarriages, stillbirths, congenital malformations and neonatal mortality rates, it was revealed that miscarriage, stillbirth, mental-motor retardation and infant mortality rates of 0-2 years were found to be higher in these marriages. Post-neonatal, infant and under-5 mortality rates were found to be higher in first-degree cousin marriages than in non-relatives. The problems faced throughout the world regarding rare diseases are also experienced in our country. These are the lack of knowledge and specialist physicians and the high cost of treatment and lack of a regulation in our country up to this day to designate rare diseases and orphan drugs. Therefore,

although there are many expert researchers working on rare diseases, the number of researchers who reach clear results is quite low. When proper working conditions are provided, when necessary arrangements are made for both researchers and patients regarding rare diseases, the diagnosis, prevention and, if possible, treatment practices can be done more properly (Dündar and Karabulut, 2010).

The Orphan Drug Study Group was founded in October 2008 with the participation of 39 companies that are members of Turkish Researcher Pharmaceutical Companies Association. The purpose of this study group is to gather the stakeholders of Rare Diseases and Orphan Drugs and to realize long-term projects, increase public awareness and contribute to national health policies. The orphan drug numbers in Turkey by numbers as determined by AIFD are detailed in the figures below (Balık, 2014).

Figure 1. Available percent of the orphan drugs licensed in the European Union in Turkey (Balık, 2014)

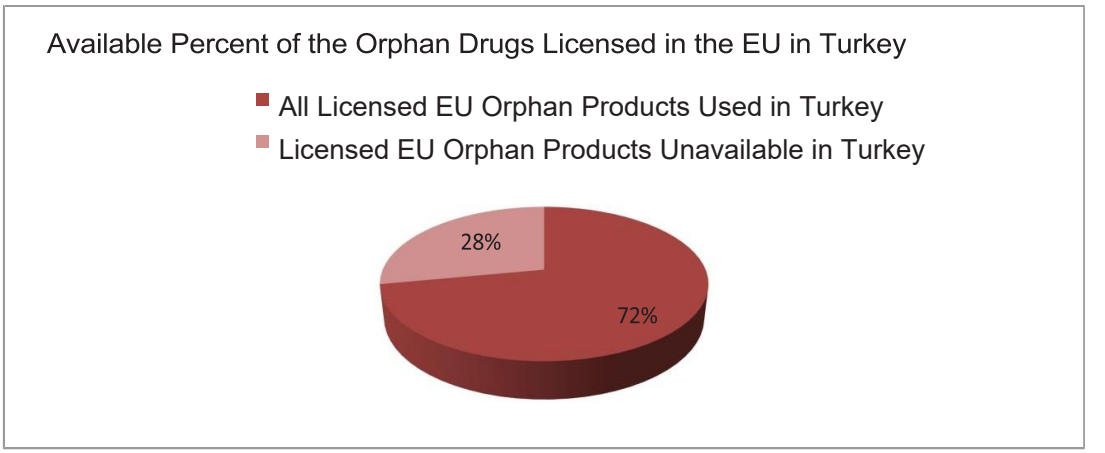


Figure 2. The percentage of the orphan medicinal products licensed in the EU used in Turkey through various access processes (Balık, 2014)

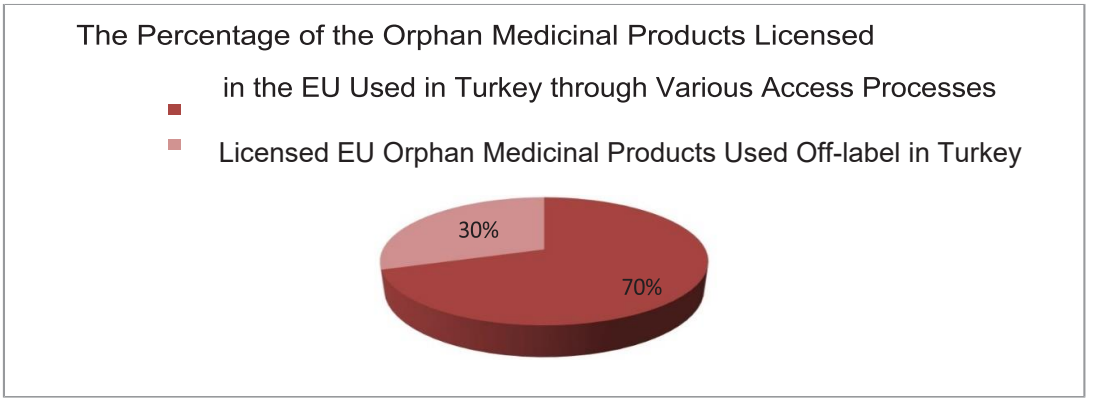


Figure 3. Therapeutic field distribution of the EU orphan products licensed in EU and used in Turkey (Balık, 2014)

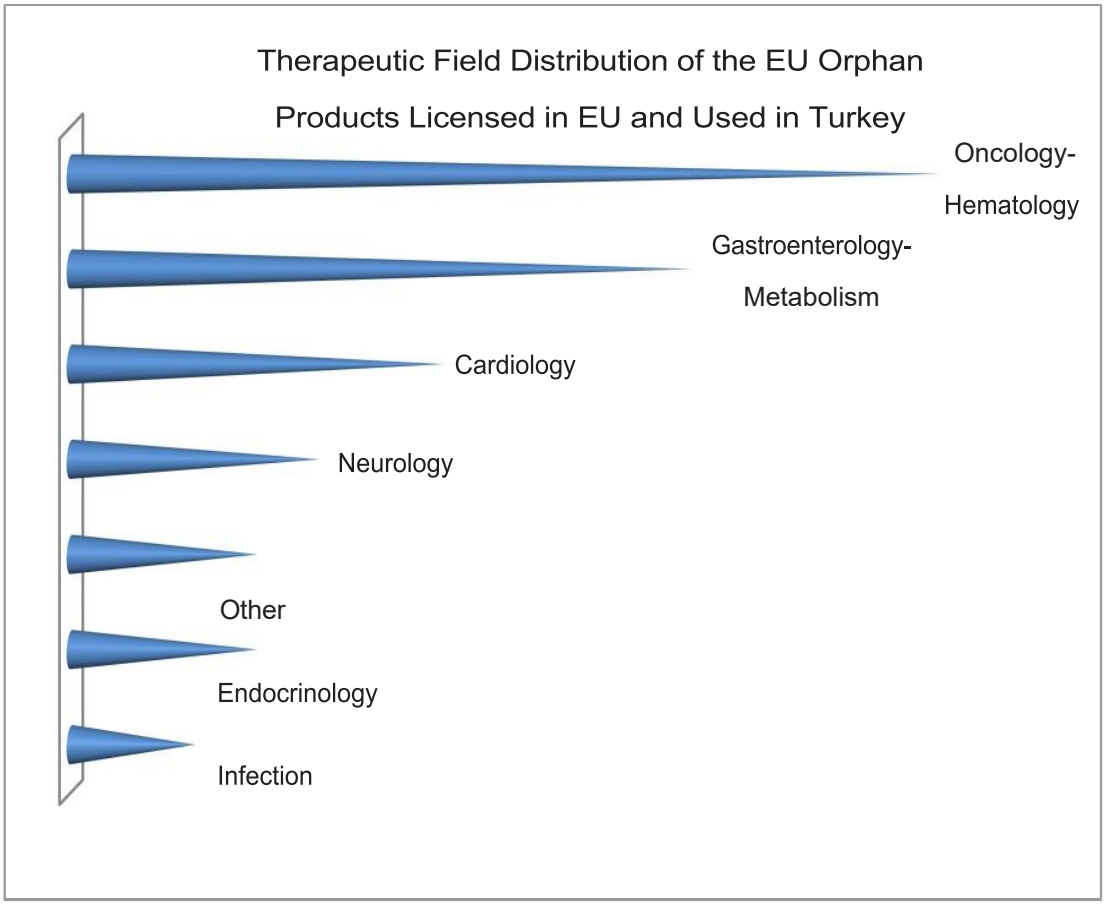
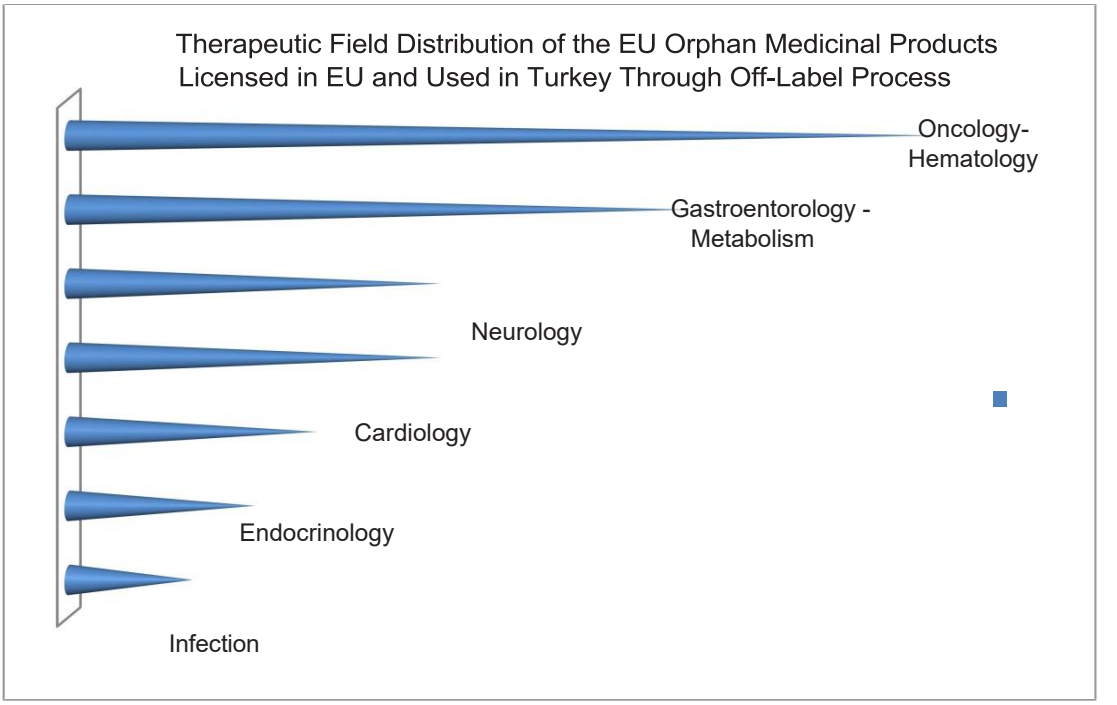


Figure 4. Therapeutic field distribution of the EU orphan medicinal products licensed in EU and used in Turkey through off-label process (Balık, 2014)



4.1. *Licensing, Pricing and Reimbursement Legislations*

Since orphan drug is not designated under the Licensing Regulation and there is no specified licensing criteria for such drugs, there is no products produced in Turkey and licensed as orphan drugs. The guideline that was started in 2011 has not been completed yet (Balık, 2014).

Pursuant to Article 7 of the Communiqué on Pricing, orphan products can be priced up to 100% of the reference price, or even up to 5% more. Additionally, the pricing can be determined according to the cost card for the orphan products produced in our country. In this case, however, the price requested on the cost card cannot exceed 20% of the reference product price. Claims of above 20% are evaluated by the Commission (Balık, 2014). However, since there is no product licensed as an orphan drug, there is no product that can use this article. There is no regulation specific to orphan drugs in the reimbursement legislation (Balık, 2014). So what are the expectations of the pharmaceutical sector on orphan drugs in Turkey? (Balık, 2014).

For licensing;

- For the Orphan Drug designation criteria to be the same as the EU, and in this context, accepting the incidence rate of the disease as 5 out of 10.000,
- Defining licensing processes that will allow easier and quicker licensing, enabling assessment priority,

- Release of GMP Audit before the application for license,
- Waiver of license application fees, analysis fees, license fees, GMP audit fees, revenue office fees, variation application fees, clinical research application fees and import application fees (Balık, 2014).

For pricing;

- Being able to determine the price of the orphan drug 5% higher than the reference price as specified in the repealed old Pricing Communiqué,
- Application of the current exchange rate on these products (Balık, 2014).

For reimbursement;

- Priority assessment of reimbursement applications for orphan drugs,
- Shortening the assessment period,
- Reduction of public discount rates considering the effect on the budget (Balık, 2014).

Conclusion

It is important to be aware that every family can catch a rare disease at any time. There has been an increase in public awareness of rare diseases in recent years but the knowledge on the development of treatment for these diseases is still quite insufficient. Therefore, regional and financial services supporting patients such as day care services, recreation centers, emergency units, socialization and rehabilitation centers, summer camps and training should be developed.

It is known that patient with rare diseases and their families are more proactive than normal patients who have other common diseases, they also have much more knowledgeable about their problems than the health professionals who are trying to relieve their suffering, because of the result of rare diseases. Therefore, the social dimension and consequences of rare diseases should be considered.

Beyond the variety of these diseases, patients suffering from rare diseases and their families face many difficulties directly caused by the rarity of these pathologies; failure to reach the correct diagnosis, lack of knowledge, social outcomes, deficiencies in health care services, high cost, limited medication, and care options.

Patients suffering from rare diseases are defined as the ones undiagnosed and untreated by the healthcare system. In this general framework of difficulties, it should be emphasized that there is always a way to use this limited but increasing information and tools available.

Conflict of Interests

Author declares no conflict of interests.

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2019 Scientific Events on Health Sciences

7th European Conference on Pharmacognosy, Phytochemistry and Natural Products
Feb 25-26, 2019
Paris, France.
https://pharmacognosy.pharmaceuticalconferences.com/

24th International meet on Pharmaceutical Biotechnology 2019
March 1-2, 2019
Paris, France
https://biotechnology.pharmaceuticalconferences.com/

16th World Congress on Industrial Pharma and Cosmetic sciences
March 01-02, 2019
Paris, France.
https://industrial.pharmaceuticalconferences.com/

BPS19 – 63th Annual Meeting of the Biophysical Society
March 2-6, 2019
Baltimore, Maryland, US
https://www.biophysics.org/2019meeting#/

3. International Conference and Exhibition on Nanomedicine and Drug Delivery
13-14 March 2019
Singapore
https://nanomedicine.pharmaceuticalconferences.com/

15. International Conference and Exhibition on Nanomedicine and Pharmaceutical Nanotechnology
18-19 March 2019
Paris, France
https://nanotechnology.pharmaceuticalconferences.com/

20th International Conference and Exhibition on Pharmaceutics & Novel Drug Delivery Systems
March 18-20, 2019
Edinburgh, Scotland
https://industrial.pharmaceuticalconferences.com/

SIDP, 19th International Congress
March 21-23, 2019
Rimini, Italy
www.sidp.it/en/Cultural-Program/2019/

AAE 2019, Annual Meeting
April 10-13, 2019
Montreal, Canada
https://eventscribe.com/2019/AAE19/index.asp

Ulusal Tıp Eğitimi Sempozyumu 2019
National Medicine Education Symposium 2019 (Language of the Symposium is Turkish)
April 10-12, 2019
Eskişehir, Turkey
http://kongre.teged.org/UTES2019

3rd ICAFOP (3. International Conference on Agriculture, Food, Veterinary and Pharmacy Sciences)
16-18 April 2019
Trabzon-Turkey
http://www.icafop.org/welcome-t1.html

International Osteology Symposium
April 25-27, 2019
Barcelona, Spain
https://www.osteology-barcelona.org/

3. Uluslararası Oral Diagnoz ve Maksillofasiyal Radyoloji Derneği Kongresi
25-28 Nisan, 2019
Belek-Antalya, Türkiye
http://odmfr2019.org/

The 5th International Mediterranean Symposium on Medicinal and Aromatic Plants
24-28 April 2019
Cappadocia – TURKEY
http://www.mesmap.com/?SyfNmb=1&pt=HOME

ACCP Updates in Therapeutics 2019
April 26-28
St. Louis, MO, USA
https://www.accp.com/meetings/abstracts.aspx

2019 ACCP Virtual Poster Symposium
May 28-29
https://www.accp.com/meetings/abstracts.aspx

European Academy of Esthetic Dentistry, 33rd Spring Meeting
May 23-25, 2019
Munich, Germany
http://munich.eaed.org/

Sepa'19-Periodontology and Oral Health Congress
May 29-June 1, 2019
Valencia-Spain
http://sepa2019.es/en/

MASCC/ISOO Annual Meeting on Supportive care in cancer
21-23 June San Francisco-USA
https://masccmeeting.org/2019#.XFwyuFwzbIU

3rd Edition of Global Conference on Pharmaceutics and Drug Delivery Systems (PDDS 2019)
24-26 June 2019
Paris-France
https://pharmaceuticsconference.com/

EEBST 2019 – VI. Ecology and Evolutionary Biology Symposium
July 10-12, 2019
Ankara, Turkey
http://eebst.org/

12th EBSA 10th ICBP-IUPAP Congress
July 20-24, 2019
Madrid, Spain
http://www.ebsa2019.com/index.php

3rd World Pharmacology Congress 2019
19-20 August 2019
Kuala Lumpur-Malasia
https://www.pharmacologyconference.org/

32nd ECNP Congress
7-10 September 2019
Copenhagen-Denmak
https://2019.ecnp.eu/

CINP International Meeting 2019
3-5 October 2019
Athens-Greece
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Instruction for Authors

Aims and Scope

The Aurum Journal of Health Sciences (AJHS - A. J. Health Sci.) is an international open access platform for basic, applied, theoretical and clinical studies in health sciences. AJHS publishes double blind peer-reviewed research articles, short reports, case reports, invited reviews and letters to the editor. AJHS is published tri-annually both in printed and electronic version. AJHS is a multidisciplinary journal on health sciences and accepts manuscripts on dental, medical, health services and pharmaceutical studies. The manuscripts linking different disciplines of health sciences will be given a priority in the journal.

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The Aurum Journal of Health Sciences publishes *research articles, short reports, reviews, case studies* on all aspects of health sciences both in electronic and printed versions. Authors are encouraged to provide proofs of their research results and/or ethical committee approvals as a supporting material which will be published electronically separately with the article.

1. *Research articles:* The manuscripts that are describing findings of an original research in regard to all aspects of health sciences will be published as a "*Research article*". The research articles should be consisting of the following parts: 1. Title; 2. Authors and affiliations; 3. Abstract; 4. Keywords; 5. Introduction; 6. Materials and Methods; 7. Results 8. Discussion; 9. Conclusion; 10. Acknowledgement;

11. Conflict of Interests; 12. References. Manuscripts submitted as a "*Research article*" do not have a wording limit. However, manuscripts that are submitted as "*Research article*" should be more than 5000 words, excluding the tables, figures and references.

2. *Short Reports:* The manuscripts that are describing preliminary findings obtained from an original research or/and results of pre-study performed on a topic in regard to all aspects of health sciences will be published as a "*Short report*". The short report articles should be consisting of the following parts: 1. Title; 2. Authors and affiliations; 3. Abstract; 4. Keywords; 5. Introduction; 6. Materials and Methods; 7. Results; 8. Discussion; 9. Acknowledgement; 10. Conflict of Interests; 11. References. Manuscripts submitted as a "*Short report*" should not exceed 5000 words excluding the tables, figures and references.

3. *Reviews:* The manuscripts that are describing critical evaluation of the current situation in the literature and providing future prospects according to current knowledge on a topic in regard to all aspects of health sciences will be published as a "*Review*". The reviews should be consisting of the following parts: 1. Title; 2. Authors and affiliations; 3. Abstract; 4. Keywords; 5. Contents; 6. Introduction; 7. Sub-Topics Provided in Contents; 8. Conclusion; 9. Acknowledgement; 10. Conflict of Interests; 11. References. Manuscripts submitted as a "*Review*" should not exceed 10,000 words excluding the tables, figures and references.

4. *Case studies:* The manuscripts that are describing a critical evaluation of an observed clinical cases in dentistry, medicine and clinical pharmacy will be published as a *"case study".* The case studies should be consisting of the following parts: 1. Title; 2. Authors and affiliations; 3. Abstract; 4. Keywords; 5. Introduction; 6. Description of the Case; 7. Discussion; 8. Acknowledgement; 9. Conflict of Interests; 10. References. Manuscripts submitted as a "*Case report*" should not exceed 5000 words excluding the tables, figures and references. A written consent of the patient may be required if the case report contains images taken from the patients. All the case reports must contain ethical committee approvals.

Preparation of manuscript and general rules

The manuscripts should be written double spaced in Arial font type and 12 pts font size. Each page should be numbered, and consecutive line numbers should be provided. Title page, authors list and affiliations should be prepared as a separate file. Tables and Figures should also be prepared as a separate file.

Title Page: The title page should contain the full title of the work which should not exceed 200 characters. Abbreviations should be avoided in the title. Main title of the manuscript should be followed by the "*short title"* which should not be longer than 70 characters. Short title should be followed by the list of author names. Author names should be given as name and surname followed by superscript Arabic numbers indicating the affiliations. One author should be designated as the corresponding author and should be indicated in the authors list with the superscript asterix symbol after the affiliation indicator. Author list should be followed by the list of affiliations which indicate the department, institution, postal code, city, country and e-mail(s) of the author(s). Finally, corresponding author full mailing address, telephone, fax and e-mail should be provided. Acknowledgement and Conflict of Interests parts should be given in the title page.

Main text: Main text should be divided into sections and sub-sections using Arabic numerals, starting from the introduction part. Sections should be indicated with bold and non-italic characters. Sub-sections should be indicated with bold and italic characters (as given in example).

Section Example: 1. Introduction

Subsection Example: 2.1. *GC-MS Analysis*

First page of the main text should contain the title followed by a 300-word abstract. Abstract should not contain citations. Abbreviations could be used in the abstract however; full explanation of the abbreviations should be given at the first time that they have appeared in the abstract. Abstract should briefly summarize the study. Abstract should contain the following information: 1. Purpose/Aim of the study; 2. Materials and methodology used in the study; 3. Key results obtained in the study; 4. Conclusion remarks. Abstract part should be followed by 6 keywords that describe the work. Keywords should be separated from one another with a semicolon.

Depending on the type of article following parts should be given in the main text. Introduction part in the manuscript should contain a brief explanation of previous studies, aim of the current study and reasoning of the study. Materials and Method part should be given in full detail allowing replication of the performed experiments/clinical studies/technical studies by other scientists. In materials and methods section all the instruments, chemicals used in the study should be explained by their brand and model. Results, should be described without any comments. Discussion and conclusion parts should not contain any speculations. A clear and concise discussion and conclusion remarks should be given.

Acknowledgement

Authors should indicate any acknowledgement related to the study in this part.

Conflict of Interests

Authors should clearly indicate any kind of conflict of interests for the study in this part. If the authors do not have any conflict of interests, they should indicate "Authors declare no conflict of interests".

Tables & Figures

The authors should indicate the position of Tables and Figures in the text by indicating the Title of the table (as given in the example). All figures should be provided as a tiff file with at least 300 dpi resolution. The images given as figures should be authentic, no manipulations should be done. Color figures are welcome in the journal and does not require a publication fee.

Example: Figure 1. ^{1}H-NMR spectrum of *Ulubelenolide*.

The figures and tables should be given as a separate file. Each table and figure given should contain a title and if required footnotes should be given. Each figure and table given should be self-explanatory.

Reference Format

Citation of references in the text

Authors must check their manuscript that every reference cited in their text should also be in the given reference list and every reference listed should be in cited in the text. Citations of unpublished results and personal communications should be avoided. Citations of literatures that were accepted by a journal and which have doi number, issue and page numbers could be cited in the text however, authors should indicate that this work is "*in press*". The citations of the web pages should be avoided. The citations in the text should adhere to the following style.

Cited reference which have a single author: (author's last name, year of publication)

Example: (Biyikoglu, 2017)

Cited reference which have two authors: (last name of the first author and second author, year of publication)

Example: (Biyikoglu and Polatoglu, 2017)

Cited reference which have three authors or more: (last name of the first author et al., year of publication)

Example: (Polatoglu et al., 2017)

Cited references which have the same first author(s) that were published in the same year: (last name of the author, year of publication and uncapitalized letters for separation)

Example: (Biyikoglu, 2017a; Biyikoglu 2017b)

Cited references as lists: The references that are going to be given as a list in a single parentheses should be first arranged alphabetically than chronologically.

Example: (Biyikoglu 2017a; Biyikoglu 2017b; Polatoglu et al., 2013)

Cited references given in text: If author names are going to be mentioned in the text for the citation than it should be given as: "….Polatoglu et al. (2013)……"

Examples: "……..Polatoglu et al. (2013) have indicated….

"……Biyikoglu (2017) demonstrated that……"

Reference formatting

The reference formatting should be given according to the following style (APA). DOI numbers should be given after the reference if available.

Reference style

Reference to a journal publication:

Polatoglu, K., Demirci, F., Demirci, B., Gören, N., Baser, K. H. C. (2010). Antibacterial activity and the variation of Tanacetum parthenium (L.) Schultz Bip. essential oils from Turkey. Journal of oleo science, 59(4), 177-184. https://doi.org/10.5650/jos.59.177

Reference to a book:

Preedy, V. R. (Ed.). (2015). Essential oils in food preservation, flavor and safety. 1st Ed., Academic Press, Elsevier, Oxford, UK.

Reference to a chapter in an edited book:

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Reference to a website:

National Cancer Institute, A success storyTaxol® (NSC 125973) https://dtp.cancer.gov/timeline/flash/success_stories/s2_taxol.htm (accessed 14 December 2017)

Reference to a Thesis:

Knight, K.A. (2011). Media epidemics: Viral structures in literature and new media (Doctoral dissertation).

Abbreviations

Full explanation of the abbreviations should be given at the first time that they have appeared in the text. Title should not contain any abbreviations. After the explanation of the abbreviations are given in the text authors could use abbreviations throughout the text.

Example: "…..Acetylcholinesterase (AChE) and butrylcholinesterase (BChE) enzymes were ……."

Chemical and Biological Nomenclature

The names of the biological organisms should be given in full of the author name at the first time they appear in the text. The genus and species names should always be written in italics. Authors could use the short name of the organism after the full name was indicated. Local names of the organisms could be mentioned however, throughout the manuscript these organisms should be referred to with their binominal names.

Chemical compounds should be preferably named according to the IUPAC nomenclature.